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Telomere length loss due to smoking and metabolic traits

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Abstract. Huzen J, Wong LSM, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, Cawthon RM, Benus GFJD, van der Horst ICC, Navis G, Bakker SJL, Gansevoort RT, de Jong PE, Hillege HL, van Gilst WH, de Boer RA, van der Harst P (University of Groningen, Groningen, the Netherlands; University of Leicester, Leicester, UK; Academic Medical Center, Amsterdam, the Netherlands; University of Utah, Salt Lake City, UT, USA; University of Groningen, Groningen, the Netherlands; and ICIN-Netherlands Heart Institute, Utrecht, the Netherlands). Telomere length loss due to smoking and metabolic traits. *J Intern Med* 2014; **275**: 155–163.

Objectives. Human age-dependent telomere attrition and telomere shortening are associated with several age-associated diseases and poorer overall survival. The aim of this study was to determine longitudinal leucocyte telomere length dynamics and identify factors associated with temporal changes in telomere length.

Design and Methods. Leucocyte telomere length was measured by quantitative polymerase chain reaction in 8074 participants from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, an ongoing community-based prospective cohort study initiated in 1997. Follow-up data were available at two time-points up to 2007. Leucocyte telomere length was measured, on between one and three separate occasions, in a total of 16 783 DNA samples. Multilevel growth models were created to identify the factors that influence leucocyte telomere dynamics.

Results. We observed an average attrition rate of 0.47 ± 0.16 relative telomere length units (RTLUs) per year in the study population aged 48 (range 39–60) years at baseline. Annual telomere attrition rate increased with age ($P < 0.001$) and was faster on average in men than in women (P for interaction 0.043). The major independent factors determining telomere attrition rate were active smoking (approximately tripled the loss of RTLU per year, $P < 0.0001$) and multiple traits of the metabolic syndrome (waist–hip ratio, $P = 0.007$; blood glucose level, $P = 0.045$, and HDL cholesterol level, $P < 0.001$).

Conclusions. Smoking and variables linked to the metabolic syndrome are modifiable lifestyle factors that accelerate telomere attrition in humans. The higher rate of cellular ageing may mediate the link between smoking and the metabolic syndrome to an increased risk of several age-associated diseases.

Keywords: ageing, biomarker, metabolic syndrome, smoking, telomeres.

Introduction

Telomeres are DNA–protein complexes at the terminal ends of linear chromosomes that consist of a large number of tandem repeats of a simple DNA sequence (TTAGGG in humans) and are capped by interacting with a protein complex (known as shelterin) [1–3]. Telomeres are essential structures involved in the protection and maintenance of chromosomal stability and are involved in cell cycle control. Telomere length shows considerable interindividual variation at birth. Both *in vitro* and *in vivo* in somatic cells, telomeres shorten progressively with repeated cell divisions and therefore with cellular replicative age. When the mean telomere length reaches a critical value, a DNA damage signal is generated, causing arrest of cell proliferation, senescence and apoptosis. Consequently, telomere length has been proposed as a marker of variation in biological ageing [1–3].

*These authors contributed equally to this study.

A number of epidemiological observations support this proposal. Although some data are conflicting, in general individuals with longer telomeres have been found to have better health and live longer than those with shorter telomeres [4–9]. An adverse association has now been shown in a considerable number of studies between shorter leucocyte telomere length and ageing-associated diseases, including cancer [8], coronary heart disease [10, 11] and heart failure [12]. For example, patients with coronary artery disease have telomere lengths comparable to those of healthy individuals who are approximately 10 years older [10, 11].

An association between leucocyte telomere length and demographic variables as well as lifestyle factors has been shown in several cross-sectional studies [3, 13, 14]. However, whether these factors affect telomere attrition has not been established; to do so would require longitudinal studies with serial measurements of telomere length in the same subjects. Therefore, in the present study, we investigated the dynamics of leucocyte telomere length in a large population-based cohort with longitudinal follow-up to identify the factors associated with temporal changes in telomere length.

Design and methods

Setting and subjects

This analysis was performed within the framework of the Prevention of Renal and Vascular End-Stage Disease (PREVEND, www.prevend.org) study. The PREVEND study is an ongoing, longitudinal, general, population-based cohort study of individuals aged 28–75 years living in the city of Groningen, the Netherlands. For the present study, we included subjects who donated a full blood sample for DNA extraction at baseline (T1) and/or during the first (T2) and/or the second (T3) follow-up visit. Details of the study have been described previously [15]. In brief, 8592 subjects completed the baseline survey (1997–1998) and were invited to visit the outpatient department after approximately 4.3 years for the first and approximately 6.6 years for the second follow-up visit. At each visit, demographic and anthropometric characteristics and serum biomarkers were assessed. The PREVEND study has been approved by the local medical ethics committee and is being conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent before enrolment.

Measurement of telomere length

Details of the methods of DNA extraction and of telomere length measurements are provided in the Appendix S1. In brief, all samples for DNA collected at different time-points were mixed and randomly extracted using a standard DNA extraction kit (QIamp, Qiagen, Venlo, the Netherlands) to neutralize potential batch effects. Mean relative leukocyte telomere length was measured using a monochrome multiplex real-time quantitative polymerase chain reaction technique (developed by R.M.C.) [16]. This technique enables the telomere-specific amplification and the single copy gene (reference) amplification to be carried out in a single reaction well with quantification measurements at different temperatures [16]. The ratio of telomere (T) to single copy gene (S) content (T/S ratio) is a relative measure of telomere length (RTL) and is expressed in arbitrary units (RTLU). All samples were measured in triplicate, and the average of the three runs was used to provide the mean RTLU for each individual. The intra-assay coefficients of variation were 2.0%, 1.9% and 4.5% for T, S and the T/S ratio, respectively.

We adhered to the arbitrary categorization of telomere trajectories as previously reported: shortening was defined as a >10% decrease in RTL, stable as a ≤10% change in RTL and elongation as a >10% increase in RTL at T3 compared to baseline [17, 18].

Other measurements and definitions

All participants completed a questionnaire regarding demographic profile and smoking habits. Smoking was categorized as current smoking, previous smoking and nonsmoking. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and categorized as follows: normal, <25 kg m^{-2}; overweight, 25–30 kg m^{-2}; and obese, ≥30 kg m^{-2}. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or the use of antihypertensive medication; prehypertension was defined as systolic blood pressure <140 and ≥120 mmHg and diastolic blood pressure <90 and ≥80 mmHg; and normotension was defined as both systolic blood pressure <120 mmHg and diastolic blood pressure <80 mmHg. Diabetes was defined as a fasting plasma glucose level of ≥126 mg dL^{-1} (7.0 mmol L^{-1}), a nonfasting plasma glucose level of ≥200 mg dL^{-1} (11.1 mmol L^{-1}) or the use of oral

antidiabetic agents. Hypercholesterolaemia was defined as a total cholesterol level of ≥250 mg dL^{-1} (6.5 mmol L^{-1}) or the use of lipid-lowering medication; ≤200 mg dL^{-1} (5.13 mmol L^{-1}) was considered an optimal cholesterol concentration. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation taking into account sex, age, ethnicity and serum creatinine levels [19]. Details of the measurement methods can be found in the Appendix S1.

Statistical analysis

To obtain a normal distribution, telomere length was natural-log-transformed. Other continuous variables with a skewed distribution (creatinine, insulin, glucose, high-sensitivity C-reactive protein, cholesterol, HDL and triglycerides) were also natural-log-transformed prior to analysis. Individuals in the bottom and top 0.5% of the RTL distribution were excluded to limit the undue influence of outliers in the regression analysis. Differences in telomere lengths between groups were tested using Student's *t*-test or one-way ANOVA. Cross-sectional associations between variables and telomere length were evaluated using standard linear regression models. Multivariate linear regression models were used to adjust for age and gender. To investigate telomere dynamics across time, two-level hierarchical growth models were constructed. Variables were centred around the grand mean (i.e. the mean was set to zero). Details of the model-building strategy, centring and multitest correction are provided in the Appendix S1.

Results

At baseline (T1), 8592 individuals participated in the PREVEND study. DNA was available for 8506 (99%) subjects, and RTL was successfully measured in 8074 subjects in the present analysis (95% of those with available DNA samples; see Fig. 1). Baseline characteristics of these 8074 subjects are presented in Table 1. The median age of our cohort at baseline was 48 (range 28–75) years (50% male, 95% Caucasian). As expected, baseline leucocyte telomere length was associated with age and gender. In addition, leucocyte telomere length at baseline was associated with ethnicity, glucose levels and diabetes, waist–hip ratio and obesity, cholesterol levels and hypercholesterolaemia, levels of HDL cholesterol, triglycerides and C-reactive protein, and cigarette smoking. Within the group of smokers, we found a dose-dependent association between the number of cigarettes smoked per day and baseline leucocyte telomere length (Tables 1 and 2). All these associations remained significant after adjustment for age and gender.

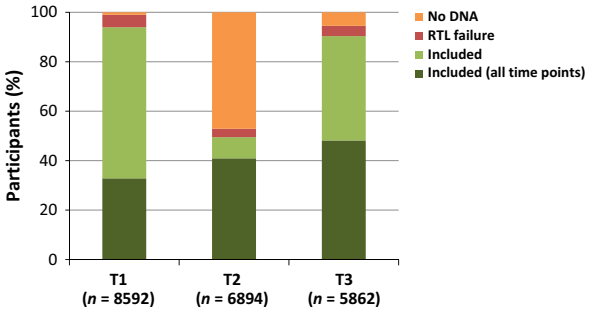

Fig. 1 *Study participants Stacked histogram of subjects showing the percentage of unavailability of DNA samples and unsuccessful RTL measurement at each time point [baseline (T1), first follow-up examination (T2) and second follow-up examination (T3)] and the percentage of subjects with successful RTL measurements at all three time-points.*

Telomere dynamics

In addition to the measurements at T1 (baseline), we obtained 8709 follow-up measurements at two follow-up time-points. At T2 (an average of 4.3 years after T1), the initiation of DNA collection was half way through follow-up of subjects. Therefore, of the remaining 6894 subjects in PREVEND, DNA was available for 3646 (53%) and RTL was successfully measured in 3412 (94%) subjects. At T3 (an average of 6.6 years after T1), 5862 participated in PREVEND, DNA was available for 5541 (95%) of the subjects and RTL was successfully measured in 5297 (96%) subjects. Overall, data were available at all three time-points for 2823 subjects, and at least one follow-up measurement in addition to the baseline measurement was obtained for 5886 subjects, who were included in the longitudinal analysis. Using the unconditional growth model, we observed an average telomere attrition rate of 0.47 ± 0.16 RTLU per year in the longitudinal analysis, which is similar to the annual attrition rate derived from our baseline cross-sectional estimates (see Table 1). RTL shortened (RTLU decrease of >10%), in 44% of subjects, was stable (RTLU change of ≤10%) in 22% and elongated (RTLU increase of >10%) in 34% after a median follow-up of 6.6 years.

Table 1 *Baseline values, and age- and gender-adjusted association with baseline telomere length*

	Baseline value (n = 8074)	B (95% CI)	Standardized B	P-value[b]
Age (years)	48 [39–60]	−0.47 (−0.52 to −0.42)	−0.21	<0.001
Male gender	4027 (49.9)	−2.51 (−1.26 to −3.76)	−0.04	<0.001
Systolic BP (mmHg)	126 [114–141]	−0.03 (−0.07 to 0.01)	−0.02	0.10
Diastolic BP (mmHg)	73 [67–80]	−0.03 (−0.10 to 0.04)	−0.01	0.43
eGFR (mL min^{-1} 1.73 m^2)	78.8 [69.9–88.4]	0.02 (−0.03 to 0.07)	0.01	0.37
Creatinine (mg dL^{-1})	0.93 [0.84–1.04]	−0.03 (−0.08 to 0.02)[a]	−0.01	0.29
Insulin (μIU mL^{-1})	1.15 [0.81–1.74]	−0.29 (−0.44 to −0.14)[a]	−0.04	<0.001
Glucose (mg dL^{-1})	84.7 [77.5–91.9]	−176.4 (−240.9 to −111.7)[a]	−0.06	<0.001
Body mass index (kg m^{-2})	25.6 [23.1–28.4]	−0.23 (−0.39 to −0.08)	−0.03	<0.01
Waist–hip ratio	0.88 [0.81–0.95]	−22.76 (−31.88 to −13.65)	−0.07	<0.001
C-reactive protein (mg L^{-1})	1.29 [0.56–2.99]	−1.82 (−2.38 to −1.26)[a]	−0.07	<0.001
Cholesterol (mg dL^{-1})	214.3 [188.8–244.0]	−157.5 (−285.7 to −29.3)[a]	−0.03	0.02
HDL cholesterol (mg dL^{-1})	49.0 [39.8–60.2]	242.5 (154.1 to 330.9)[a]	0.07	<0.001
Cholesterol–HDL ratio	4.36 [3.36–5.63]	−5.38 (−7.25 to −3.51)[a]	−0.07	<0.001
Triglycerides (mg dL^{-1})	102.7 [75.2–149.6]	−260.2 (−369.9 to −151.3)[a]	−0.05	<0.001
Cigarettes/day	447 (16.4) | 1786	−2.20 (−3.37 to −1.03)		<0.001
1–6 | 6–20 | >20	(65.4) | 499 (18.3)			

Data are presented as median [interquartile range] or number (percentage).
BP, blood pressure; eGFR, estimated glomerular filtration rate.
[a]Beta estimate is for 1 natural-log-transformed unit increase.
[b]P-value after adjustment for age and gender.

A basic growth model consisting of baseline telomere length, gender, age and the age × gender interaction term was constructed (Table 3). Ethnicity was considered but not included in the basic model because inclusion did not lead to any improvement (Table S1, Appendix S1). Telomere attrition rate increased with advancing age, and this effect was more pronounced in men than in women (P for interaction 0.043; Table 3). This basic model was then used to evaluate the other variables shown in Tables 1 and 2. All variables that caused a significant ($P < 0.05$) improvement when added to the basic model are presented in Table 3. Both higher systolic ($P = 0.038$) and diastolic ($P = 0.031$) blood pressures but not hypertension were related to an increased telomere attrition rate. Similarly, serum triglyceride level ($P < 0.001$) but not hypercholesterolaemia or total cholesterol was related to an increased rate of telomere attrition, and higher HDL cholesterol levels were protective ($P < 0.001$). Finally, other traits related to the metabolic syndrome, including the presence of diabetes ($P = 0.022$) and higher waist–hip ratio ($P < 0.001$) and active smoking were related to accelerated telomere attrition ($P < 0.001$). In the longitudinal analysis, there was no dose-dependent effect of smoking. The inflammatory marker C-reactive protein was correlated with baseline telomere length but was not a significant determinant when added to the basic growth model in the longitudinal analysis.

Multifactorial model

All variables that showed an effect when added to the basic model were evaluated together to test which had the greatest independent effects on telomere attrition rate. Nonsignificant variables were removed using a stepwise backward strategy (excluded variables and their P-values are presented in Table S2, Appendix S1). In the final multifactorial model, higher glucose levels, higher waist–hip ratio, lower HDL cholesterol and active smoking were identified as the major independent modifiable factors explaining an increased telomere attrition rate (Table 4 and Fig. 2). For example, the annual attrition rate of a female, nonsmoking subject with average values for

Table 2 *Baseline RTL amongst subgroups*

	n (%)	Median T/S (IQR)	*P*-value[a]	Adjusted *P*-value[b]
Gender				
Male	4027 (50)	0.98 (0.82–1.20)	<0.001	<0.001
Female	4047 (50)	1.03 (0.84–1.25)		
Ethnicity				
Caucasian	7664 (95)	0.99 (0.83–1.22)		
Black	77 (1)	1.11 (0.91–1.44)	0.01	0.02[c]
Asian	167 (2)	1.02 (0.85–1.30)	0.06	0.33[c]
Other	166 (2)	1.07 (0.84–1.28)	0.07	0.29[c]
Hypertension				
Yes	2577 (32)	0.95 (0.79–1.14)	<0.001	0.01
Prehypertension	3241 (40)	1.03 (0.84–1.24)		
No	2256 (28)	1.04 (0.87–1.29)		
Diabetes				
Yes	203 (3)	0.92 (0.76–1.11)	<0.001	0.02
No	7871 (98)	1.01 (0.83–1.23)		
Obesity				
Yes	1259 (16)	0.96 (0.80–1.16)	<0.001	<0.01
Overweight	3255 (40)	0.98 (0.81–1.21)		
No	3473 (43)	1.04 (0.86–1.27)		
Hypercholesterolaemia				
Yes	1682 (21)	0.96 (0.80–1.16)	<0.001	<0.01
Borderline	3713 (46)	0.99 (0.82–1.22)		
No	2679 (33)	1.05 (0.87–1.28)		
Smoker				
Yes	2742 (34)	0.98 (0.81–1.20)	<0.001	<0.001
Former	2929 (36)	0.99 (0.83–1.22)		
No	2373 (29)	1.04 (0.86–1.27)		

IQR, interquartile range.
[a]*P*-value for difference between groups.
[b]Adjusted for age and gender.
[c]Compared with Caucasians.

baseline telomere length, age, glucose, waist–hip ratio and HDL cholesterol was 0.26 RTLU (equal to the estimate for 'follow-up time' as all other variables in the model are 0). However, if instead this same subject was a smoker, the estimated attrition rate would be 0.94 RTLU (0.26 plus an additional 0.68 for smoking). In the case of a female smoker with a glucose level one standard deviation higher and HDL cholesterol level one standard deviation lower than average, the estimated attrition rate would rise to 1.33 RTLU (0.68 plus 0.13 for glucose and 0.26 for HDL cholesterol).

Discussion

Here, we describe the first comprehensive evaluation of the determinants of leucocyte telomere attrition in humans. Our principal findings are that leucocyte telomere attrition shows wide variation between individuals, with mainly shortening but also elongation, and is associated with parameters of the metabolic syndrome and smoking. Although baseline leucocyte telomere length was the principal determinant of RTL change, we identified age, gender, glucose levels, waist–hip ratio, HDL cholesterol and smoking as factors that also

Table 3 *Estimates of annual leucocyte telomere attrition rate*

Model	Variables included in model	Beta estimate (95% CI)	Standardized beta estimate (95% CI)	*P*-value
Unconditional	Follow-up time (years)	0.47 (0.31 to 0.64)		<0.001
Basic model	Follow-up time (years)	0.22 (0.05 to 0.39)		0.011
	Baseline telomere length (RTLU)	10.54 (10.21 to 10.87)	3.97 (4.09 to 3.84)	<0.001
	Age (per 10 years)	0.52 (−0.37 to 0.67)	0.88 (1.04 to 0.71)	<0.001
	Gender[a]	0.20 (0.04 to 044)	0.12 (0.24 to 0.01)	0.097
	Age × gender interaction[a]	0.21 (0.01 to 0.41)	0.12 (0.25 to 0.01)	0.043
Blood pressure	Systolic (per 10 mmHg)	0.07 (0.01 to 0.14)	0.15 (0.29 to 0.01)	0.038
	Diastolic (per 10 mmHg)	0.15 (0.01 to 0.29)	0.14 (0.27 to 0.01)	0.031
Metabolic factors	Insulin (μIU mL^{-1})[b]	0.22 (0.01 to 0.42)	0.13 (0.26 to 0.01)	0.039
	Glucose (mg dL^{-1})[b]	1.29 (0.58 to 2.01)	0.22 (0.35 to 0.10)	<0.001
	Diabetes	0.88 (1.63 to 0.13)	0.12 (0.02 to 0.24)	0.022
	Body mass index (kg m^{-2})	0.04 (0.01 to 0.06)	0.14 (0.26 to 0.02)	0.013
	Waist–hip ratio (per 0.1 ratio)	0.41 (0.23 to 0.59)	0.37 (0.54 to 0.21)	<0.001
	HDL cholesterol (mg dL^{-1})[b]	−1.26 (−0.82 to −1.71)	−0.37 (−0.24 to− 0.50)	<0.001
	Triglycerides (mg dL^{-1})[b]	0.50 (0.27 to 0.74)	0.27 (0.39 to 0.14)	<0.001
Smoking	Smoking[c]	0.73 (1.03 to 0.43)	0.29 (0.41 to 0.17)	<0.001

[a]Male = 1, Female = 0.
[b]Beta estimate is for 1 natural-log-transformed unit increase.
[c]Compared to nonsmokers.

Table 4 *Estimates of annual leucocyte telomere attrition rate in the multifactorial model*

Variables included	Beta estimate (95% CI)	Standardized beta estimate (95% CI)	*P*-value
Follow-up (years)	0.26 (0.02 to 0.53)		<0.001
Baseline telomere length (RTLU)	10.70 (10.37 to 11.04)	4.03 (4.15 to 3.90)	<0.001
Age (per 10 years)	0.48 (0.32 to 0.64)	0.79 (0.96 to 0.61)	<0.001
Gender[a]	0.31 (0.01 to 0.63)	0.15 (−0.01 to 0.32)	0.06
Age × gender interaction[a]	0.21 (0.01 to 0.42)	0.11 (0.31 to 0.01)	0.04
Glucose (mg dL^{-1})[b]	0.76 (0.02 to 1.51)	0.13 (0.27 to 0.01)	0.04
Waist–hip ratio (per 0.1 ratio)	0.27 (0.07 to 0.46)	0.25 (0.43 to 0.07)	<0.01
HDL cholesterol (mg dL^{-1})[b]	−0.91 (−0.44 to −1.39)	−0.26 (−0.12 to −0.40)	<0.001
Smoking[c]	0.67 (0.37 to 0.98)	0.27 (0.39 to 0.15)	<0.001

[a]Male = 1, Female = 0.
[b]Estimate is for 1 natural-log-transformed unit increase.
[c]Compared to nonsmokers.

have a substantial effect. For example, the effect of active smoking increased the yearly attrition rate by more than threefold. Subjects who stopped smoking before entering the study still had shorter baseline leucocyte telomere length, although their annual attrition rate was comparable to that of subjects who never smoked.

Based on cell cultures and cross-sectional measurements, it has been assumed that telomere length of somatic cells can only become shorter over time. However, this view has recently been challenged by the findings of several studies measuring leucocyte telomere length on multiple occasions [17, 20, 21]. Our data also support the notion

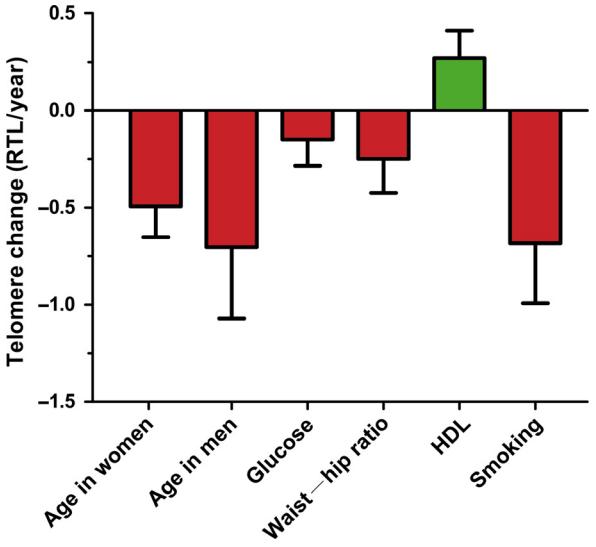

Fig. 2 *Effect size for RTL change in the final model. The effects of the variables present in the full model on RTL change. The 0-line represents the basic attrition rate for nonsmoking subjects when all other centred variables are 0 (mean). The boxes represent the additional telomere attrition rate for an increase of 10 years for women, 10 years for men and one standard deviation of glucose concentration, waist–hip ratio and HDL level, respectively. For smoking, the box represents the additional telomere attrition for current smokers. The whiskers represent the 95% confidence interval for the estimates.*

of a dynamic pattern including shortening and lengthening of leucocyte telomere length over time. The time course of these fluctuations is currently unknown as they were observed after a relatively long follow-up period of 5–10 years with few measurement time-points. In a recent small-scale study, a pattern of shortening and lengthening was also observed over a period of only 12 months, suggesting an oscillating telomere length over time [22]. Despite the observed dynamic pattern of leucocyte telomere length, we confirmed that the mean RTL of our population shortened over time. We believe our observed dynamic pattern of telomere length of circulating leucocytes is consistent with the hypothesis of Chen *et al.* [23] that 'age dependent shortening is the rule'. The mechanism for this presumed oscillating telomere length of the circulating leucocyte pool remains unclear; differences in cell fractions or true biological effects, such as transient telomerase activation, have been suggested [24, 25].

In cross-sectional studies, associations have been shown between telomere length and age as well as several other factors including ethnicity [13], smoking [14], hypertension [26], body mass index [14, 26], waist–hip ratio [17],and levels of insulin [7, 26], glucose [26], total cholesterol [27], HDL cholesterol [28], triglycerides [27] and C-reactive protein [7]. However, it remains unclear whether these factors cause leucocyte telomere attrition. Here, we observed the same cross-sectional associations at baseline but have demonstrated that glucose levels, waist–hip ratio, HDL cholesterol and smoking have a substantial independent effect on longitudinal leucocyte telomere attrition. Some factors, such as blood pressure traits, did not remain significant after inclusion of multiple stronger traits, which might be attributed to colinearity with stronger metabolic traits or lack of power to detect smaller influences. In addition, we cannot exclude the presence of bias related to certain traits. For example, we have insufficient information to study the potential unrecorded bias of blood pressure-lowering pharmacotherapy. Our observation that leucocyte telomere attrition rate is associated with baseline telomere length, gender and waist–hip ratio confirms the findings of previous studies [17, 20, 21]. Because we modelled the effect of time instead of cross-sectional data, we add the knowledge that higher age is associated with a higher leucocyte telomere attrition rate. Further novel findings are the associations between leucocyte telomere attrition rate and both blood glucose levels (negative association) and HDL cholesterol (positive association). The mechanisms linking these factors to faster leucocyte telomere attrition rates are currently unclear, although levels of oxidative stress and/or inflammation are likely to have a role. It is interesting that low-grade inflammation, as assessed by the level of high-sensitivity C-reactive protein, did not affect leucocyte telomere attrition rate. This is in line with an earlier observation, but it should be recognized that this does not exclude the possibility of an association with low-grade inflammation as assessed by other markers [29]. Another possibility is that oxidative stress could provide a molecular mechanism for telomere attrition [30]. A common denominator of higher glucose levels [31], higher waist–hip ratio [32] and smoking [33] is increased levels of oxidative stress. HDL, on the other hand, is known to have antioxidative properties [34]. It has also been suggested that HDL signalling pathways intersect with key stress responses and survival pathways, thereby modulating the ageing process [35]. A potential target of HDL signalling pathways is c-Jun N-terminal

kinase, which can be activated by several triggers, including HDL, and functions as a potent protective factor in flies [36].

Our findings should be interpreted in the context of several limitations. The main limitation is that we studied mean leucocyte telomere length, which consists of a mixture of different leucocyte subsets with different individual telomere lengths and does not necessarily translate to other tissues. Secondly, our study was conducted in a primarily Caucasian cohort, and therefore, our findings may not extend to other ethnic populations. Thirdly, we have insufficient information about some factors that are associated with healthy ageing, such as physical activity [37] and dietary habits [18]. Finally, we were not able to provide a molecular mechanism to explain the reported observations.

Nevertheless, it is of great interest that, besides age and gender and known genetic factors [38, 39], the factors found to influence leucocyte telomere length are modifiable and thus present potential targets to decrease leucocyte telomere shortening. In a recent study in telomerase-deficient mice, it was demonstrated that telomerase reactivation through knock-in of telomerase eliminated degenerative phenotypes across multiple organs [40]. These findings suggest that preventing leucocyte telomere shortening might have important clinical implications in the prevention of multiple age-related diseases. One could postulate that modifying smoking behaviour and metabolic traits will lead to a slower pace of biological ageing. This might also account for other factors not investigated in the present study; for example, blood levels of omega-3 fatty acids [18] and vigorous physical activity [37] have both been suggested to decelerate leucocyte telomere attrition. Future studies should address whether interventions aimed at modifying telomere biology indeed preserve or even increase telomere length and promote healthy ageing.

In conclusion, we present the results from the largest study measuring leucocyte telomere length to date and provide novel evidence for a longitudinal association between accelerated telomere loss and several factors: high blood glucose levels, increased waist–hip ratio, reduced blood HDL cholesterol levels and smoking. This finding supports the hypothesis that modifiable lifestyle factors influence the pace of biological ageing and provides a mechanistic link to an increased risk of numerous age-associated diseases. Whether altering telomere dynamics could impact on healthy ageing requires further confirmation.

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Conflict of interest statement

The authors have no conflict of interests to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary Appendix.